Morphofunctional Changes in Hepatopancreatobiliary Organs in Experimental Dyslipoproteinemia

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Dyslipoproteinemia was induced and corrected in 40 Chinchilla rabbits. Lipid metabolism disorders were corrected by Fishant vaseline-pectin emulsion and partial ileoshunting. Experimental dyslipoproteinemia initiated destructive dystrophic processes in the hepatopancreatobiliary organs manifesting by liver stenosis, gallbladder cholesterosis, and focal structural and functional disorders in pancreatic parenchyma. Correction of dyslipoproteinemia normalized the status of hepatopancreatobiliary organs, decreased the severity of destructive processes, and prevented their further development.

Key Words: *dyslipoproteinemia; pancreas; liver; gallbladder; spleen*

Dyslipoproteinemia is a key component in the pathogenesis of atherosclerosis and its complications. Moreover, systemic metabolic background in dyslipoproteinemia modifies functions of many organs. The integrating effect of dyslipoproteinemia on the organism can be evaluated within the framework of lipid distress syndrome. The diagnosis of this syndrome includes investigation of not only lipid spectrum of blood plasma, but also the function of the reticuloendothelial system, relationships between the host and autochtonic microflora, and organic symptoms (involvement of target organs) [3].

However changes in the liver, gallbladder, pancreas, and spleen in lipid distress syndrome are little studied [5,7,8].

We investigated the morphological and functional changes in the hepatopancreatobiliary organs in experimental dyslipoproteinemia (EDLP).

MATERIALS AND METHODS

The study was carried out on 40 Chinchilla rabbits (3.1± 0.1 kg) fed granulated fodder with crystalline cholesterol (0.3 g/day/kg) for inducing EDLP modeling.

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Total plasma cholesterol, triglycerides, HDL, LDL, and VLDL cholesterol were measured before the experiment and after 1 and 2 months, and dyslipoproteinemia coefficient was estimated. Lipid catabolizing function of the liver was evaluated by the lipid curve after fat load as described previously [11]: the animals were fed 20 g sunflower oil after overnight fasting and plasma triglycerides were measured before and 5, 9, 13, 24, and 29 h after fat load.

One month after induction of EDLP partial ileoshunting (PIS) by Buchwald—Savel'ev's method with the creation of a terminolateral ileoanastomosis was performed in 12 animals (group 1). In group 2 (n=12) EDLP was corrected by Fishant vaseline-pectin emulsion (VPE) in a dose of 20 g twice a week; no correction of EDLP was carried out in group 3 (n=16).

After 2 months all animals were sacrificed by injection of a lethal barbiturate dose and liver, gallbladder, pancreas, and spleen specimens were collected. For morphological analysis the specimens were fixed in 10% neutral formalin. Some specimens were embedded in paraffin and 5-7-μ sections were stained with hematoxylin and eosin. Other specimens (except specimens of the spleen) were used for histochemical analysis: the content of lipids (Sudan III staining) and cholesterol (Schultz' method) was measured on cryostat sections.

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RESULTS

After 4-week cholesterol diet, plasma content of cholesterol in groups 1, 2, and 3 increased 10-12 times, of triglycerides 5-6-fold, LDL 16-17-fold, VLDL 4-5-fold, and dyslipoproteinemia coefficient increased 4-5-fold in comparison with the initial and control values (Table 1). Changes in lipid metabolism corresponded to type II and IV dyslipoproteinemia [6]. Body weight at the end of the experiment did not differ from the initial in all groups. Parameters of lipid metabolism did not change in control animals.

Triglyceride clearance significantly decreased after 1 month of atherogenic diet. Parameters of lipid curves in experimental groups were similar and significantly surpassed the control values ($p<0.05$, Fig. 1, *a*), which attested to impaired lipid-catabolizing capacity of apoB/E receptors in the liver [13,14], *i. e.* depression of the reticuloendothelial function [8,12].

Correction of EDLP by PIS and injection of VPE improved lipid metabolism parameters. In the PIS group, plasma cholesterol, triglycerides, HDL, LDL, and VLDL returned to normal (Table 1). Dyslipoproteinemia coefficient was 1.1±0.2, which did not differ significantly from the initial value ($p>0.05$).

In VPE-treated rabbits, cholesterol content significantly decreased in comparison with that at the end of the first month of atherogenic diet, but still 3-fold surpassed the initial level (Table 1). Plasma triglycerides and HDL returned to normal by the end of the second month; the levels of LDL and VLDL also tended to decrease. The dyslipoproteinemia coefficient did not differ significantly from the initial and control values.

Extrapolation of previous clinical data on long-term VPE therapy to the results of the present experiment suggests that longer correction of dyslipoproteinemia with this agent will further decrease the level of atherogenic lipids [2].

In group 3 (without correction) plasma concentration of cholesterol increased 20-22 times, triglycerides 10-11 times, LDL 22-25 times, and VLDL 8-10 times. Dyslipoproteinemia coefficient increased 4-5-fold in comparison with the initial data and control.

One month after PIS, parameters of lipid curve returned to normal and virtually did not differ from the control. Plasma triglyceride concentration in this group decreased to the initial level after 9 h. Positive changes in the 24-h curve of plasma triglyceride concentrations were observed in animals treated with VPE. Absolute plasma triglyceride concentrations still surpassed the normal, but were lower than before treatment and approximated the control values (Fig. 1, *b*).

The shape of lipid curve in animals fed atherogenic diet without correction did not change, while the absolute values increased (Fig. 1, *b*). In the control, daily changes in triglyceride concentrations after fat load did not change in comparison with the initial time course of these values.

Hence, PIS and conservative blockade of enterohepatic circulation of bile acids by VPE effectively correct EDLP by restoring the capacity of apoB/E-receptors to capture chylomicron remnants from the portal blood flow and normalizing the function of the liver reticuloendothelial system [10,11].

The liver underwent the most pronounced pathological structural and functional transformations in EDLP. Local structural changes in hepatic lobules

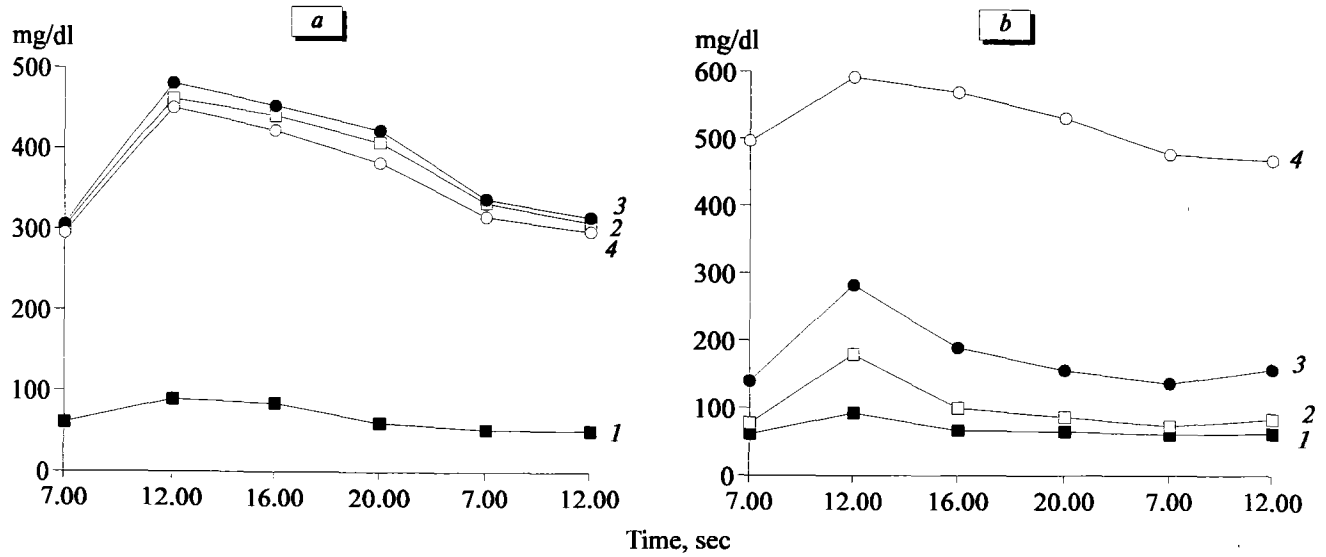

Fig. 1. Daily fluctuations of plasma triglyceride concentrations in rabbits with experimental dyslipoproteinemia. *a*) before correction; *b*) after correction.

caused by mosaic changes in the hepatocyte population were seen in all liver preparations (Fig. 2, *a*). Signs of degeneration were seen in many hepatocytes: enlargement and deformations combined with decreased basophilia, vacuolization, or subtotal clarification of the cytoplasm. Some cells looked like hollow structures with ectopic pyknotic nucleus, which reflected parallel development of destructive processes.

Groups of necrobiotic hepatocytes were often seen in hepatic cords. Variability of cell structure in epithelial laminae together with hypertrophy of stellate macrophages, local dilatation of biliary capillaries, and stenosed sinusoidal lumen destroyed the composition of liver cords. Microscopic analysis of reticuloendothelial cells showed not only enlargement, but also hyperplasia of Kupffer's cells in EDLP (Fig. 2, *b*). Activation of macrophages can be due to resorption of lipid stores as a typical adaptive reaction to EDLP [10].

It should be emphasized that pathological structural transformations in the liver were mainly located periportally and only few of them were found in the central zones of hepatic lobules. Hepatocyte degeneration was preceded by mesenchymal reactions most pronounced in the portal tract zones, where focal round-cell infiltrations were found (Fig. 2, *c*). Some preparations contained mast cells and individual polymorphonuclears. Small clusters of hematogenic elements were more often seen in the parenchyma and rarely in the center of lobules.

Histochemical analysis demonstrated that signs of liver degeneration in EDLP detected by general morphological study are caused by fatty hepatosis. Fatty infiltration of the liver parenchyma was topologically identical to degenerative process described above. Small-droplet (in some cells large-droplet) steatosis was observed in periportal hepatocytes, and extracellular fatty cysts were sometimes seen in the parenchyma.

Disperse lipid accumulation was seen in hepatocytes of centrolobular zones; unlike the periphery, these accumulations presented as focal (disseminated or zonal) steatosis.

Structural alterations in the gallbladder wall in EDLP were less pronounced. The relief of its mucosa was characterized by alternation of zones with intense plication and smooth areas. Small foci of lymphocytic macrophagal infiltration were detected in the lamina propria, while absorbed amorphous eosinophilic material was seen on the epithelium surface. Sudan III staining showed lipid-loaded macrophages in the subepithelial zone of the bladder wall (Fig. 2, *e*). Sudanophilic substance was detected in the cytoplasm and on the surface of individual epitheliocytes.

Histochemical study showed focal depositions of the reaction product with cholesterol in the epithelium and lamina propria of the gallbladder mucosa, which were more abundant than in the liver.

Morphological changes in rabbit pancreas in EDLP can be classified as reactive restructuring of its parenchyma manifested in discomplectation of acini, decreased basophilia and reduced zonal polarization of exocrinocyte cytoplasm, dilatation of excretory ducts in comparison with the control (Fig. 3, *a*). These morphological changes can be regarded as impairment of the typical asynchronous regimen of acinar cell functioning. Signs of progressive necrobiosis were detected in some sites of the pancreas: the acini lost their characteristic shape and looked like clusters of small cells with homogenous cytoplasm and pyknotic nuclei (Fig. 3, *b*).

Round-cell infiltrations, increased number of adipocytes, and individual dilated ducts (including intral-

TABLE 1. Lipid Metabolism in Rabbits with EDLP and Its Correction by Different Methods (mg/dl, $M \pm m$)

Parameter	Control (n=16)	Experiment				
		initial	after 1 month	after 2 months		
				PIS (n=12)	VPE (n=12)	without correction (n=16)
Cholesterol	30.6±1.4	39.5±6.9	421.3±37.2	45.1±13.2*+	118.6±18.3*+	683.2±171.5*+
Triglycerides	61.1±1.3	58.4±12.6	305.7±2.3	77.8±12.7*+	13.9±21.7	496.4±135.1*+
HDL	14.0±0.6	14.3±3.2	76.9±13.1	21.3±5.8*+	42.9±7.6*+	126.2±33.4*+
LDL	6.7±2.3	14.9±6.9	281.8±26.3	8.1±2.4*+	47.4±8.3*+	464.8±101.2*+
VLDL	12.3±0.4	12.5±0.7	53.0±11.6	16.2±4.1	30.1±5.2*+	95.7±25.8*+
Dyslipoproteinemia coefficient	1.2±0.2	1.8±0.5	4.5±0.2	1.1±0.2	1.8±0.3	4.5±0.3*+

Note. $p<0.05$: *vs. control, +vs. initial value. Initial values and values after 1 month are shown only for 1 group of animals; the data in other groups were similar.

obular) with desquamated epithelium were often observed in the interlobular stroma and rarely in the glandular parenchyma (Fig. 3, *c*).

Pathomorphological picture evidenced impaired structure of the pancreas in EDLP and can be regarded as an objective prerequisite for the formation of chronic pancreatitis [1].

Signs of hyperplastic processes in the reticuloendothelial stroma of the spleen were detected in animals with EDLP (Fig. 3, *d*).

Morphological studies demonstrated a possibility of regression of EDLP-induced changes in the hepatobiliary organs caused by PIS and VPE. Normal structure of hepatic cords was restored in the majority of lobules, degenerative processes become less extensive and pronounced, and signs of activation of Kupffer's cell appeared. In some lobules the effect of treatment was less pronounced (signs of steatosis persisted), but only disperse (rarely small-droplet) lipid accumulations were seen (Fig. 2, *d*) against the background of weak

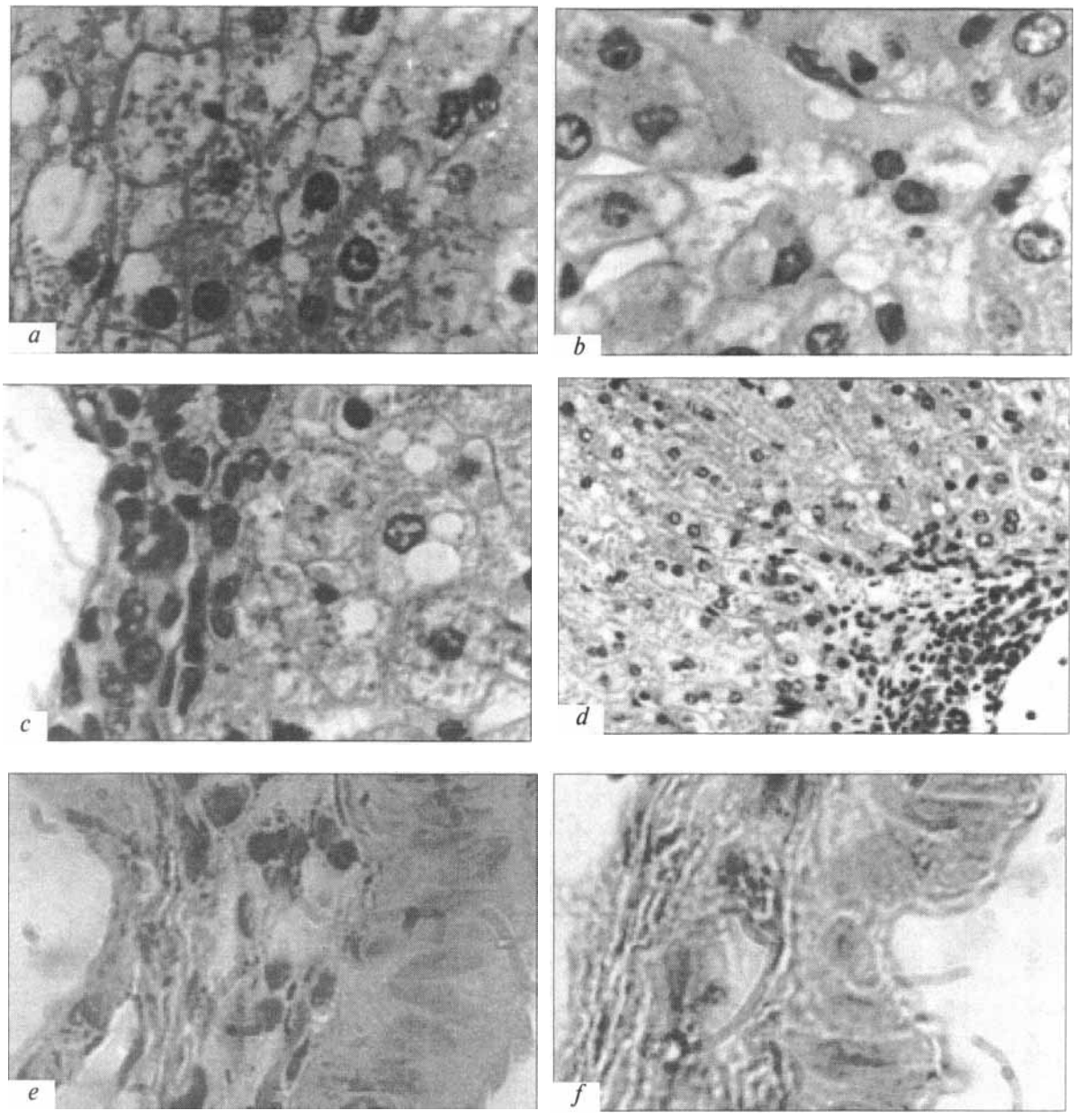

Fig. 2. Histological examination of liver and gallbladder specimens in experimental dyslipoproteinemia. Staining with hematoxylin andeosin (*a-d*), by Schultz' method (*e*), and with Sudan III (*f*), ×480 (*a-c, e, f*), ×120 (*d*). *a*) small- and large-droplet vacuolization of hepatocyte cytoplasm; *b*) paravasal interstitium edema, changed structure of the central vein, and hypertrophic stellate macrophages in the liver; *c*) cell infiltration in the stroma of portal tract; *d*) rabbit liver 1 month after partial ileoshunting. Focus of disperse fatty infiltration of liver parenchyma; *e*) focal deposition of cholesterol in the gallbladder mucosa; *f*) rabbit gallbladder 1 month after partial ileoshunting. Reduced "cholesterol incorporations" in the mucosa.

macrophagal reaction and mononuclear infiltration of the interstitium around periportal tracts. There was virtually no round-cell infiltration in the parenchyma, vascular disorders and destructive changes in hepatocytes virtually disappeared. Connective tissue growth was observed around some portal tracts, which indicated healing of destruction foci.

Correction by both methods restored typical relief of the gallbladder mucosa and reduced lipid saturation of cell elements (Fig. 2, *f*) and macrophage activity.

After normalization of lipid metabolism, the morphological picture of the pancreas indicates virtually complete recovery of the organ structure irrespective of the method of treatment (Fig. 2, *f*). Signs of fatty necrobiosis of glandular cells disappeared, the structure of these cells and excretory ducts and the architectonics of the local microcirculatory bed returned to normal. However, sites of degeneration were still present in the parenchyma.

Moderate reversion of the reticuloendothelial hyperplasia was detected in spleen preparations; signs of antigenic stimulation disappeared.

Hence, EDLP leads to the development of destructive structural and functional changes in the pancreas, morphologically classified as chronic lipogenic pancreatitis. A characteristic feature of this pathology (apart from changes in the pancreas) is obligatory involvement of the hepatobiliary system, which manifests by the development of liver steatosis, gallbladder cholesterosis, and specific reaction of reticuloendothelial macrophages in the spleen. Conservative and surgical blockade of enteropathogenic circulation of

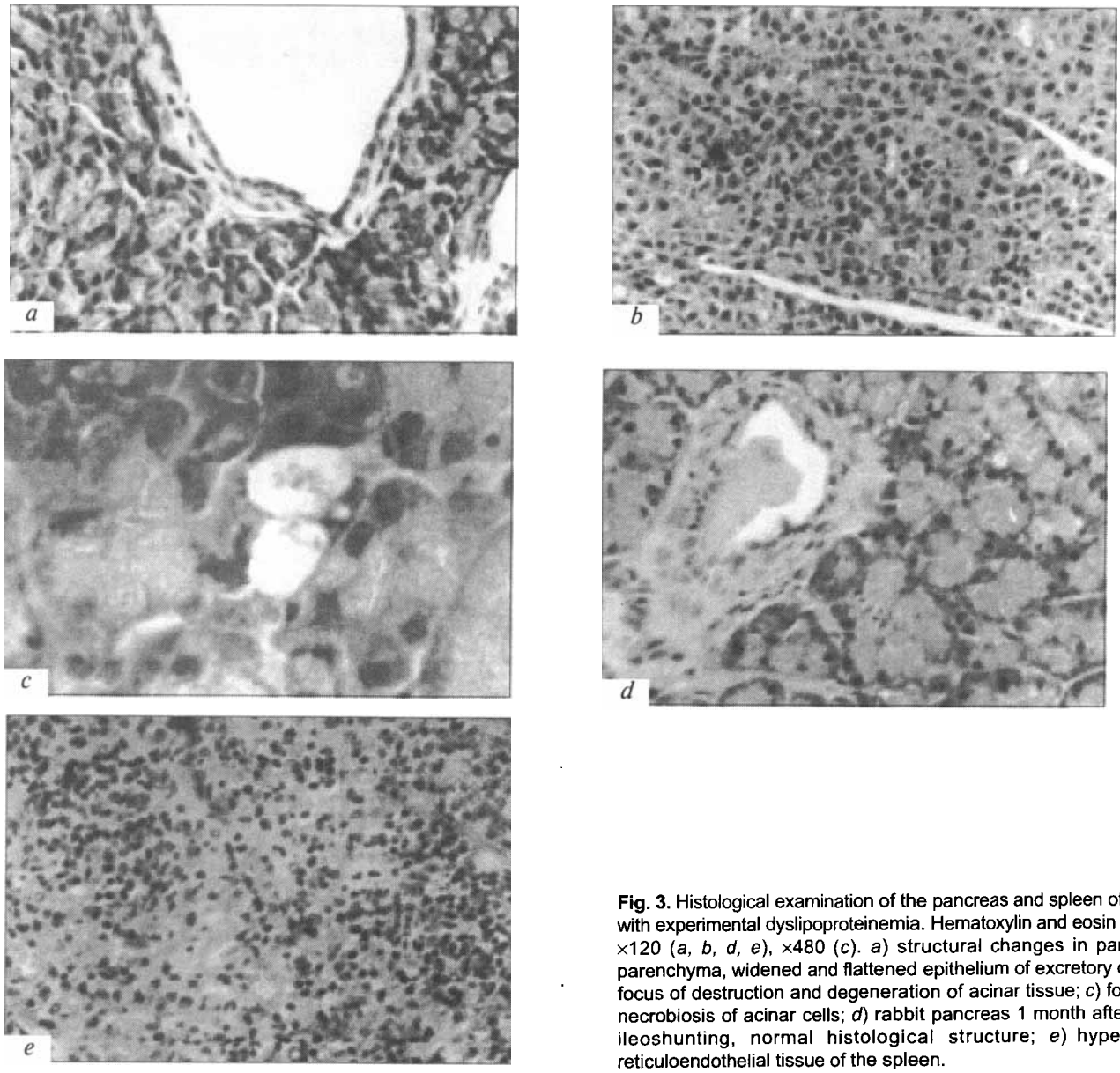

Fig. 3. Histological examination of the pancreas and spleen of a rabbit with experimental dyslipoproteinemia. Hematoxylin and eosin staining, ×120 (*a, b, d, e*), ×480 (*c*). *a*) structural changes in pancreatic parenchyma, widened and flattened epithelium of excretory ducts; *b*) focus of destruction and degeneration of acinar tissue; *c*) focal fatty necrobiosis of acinar cells; *d*) rabbit pancreas 1 month after partial ileoshunting, normal histological structure; *e*) hyperplastic reticuloendothelial tissue of the spleen.

bile acids by PIS and injection of VPE normalized lipid metabolism and corrected the pathological processes in the liver, gallbladder, and pancreas.

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